

NOTE

Mosquitoes feeding on caterpillars of the Common Buckeye butterfly, *Junonia coenia* (Lepidoptera: Nymphalidae)

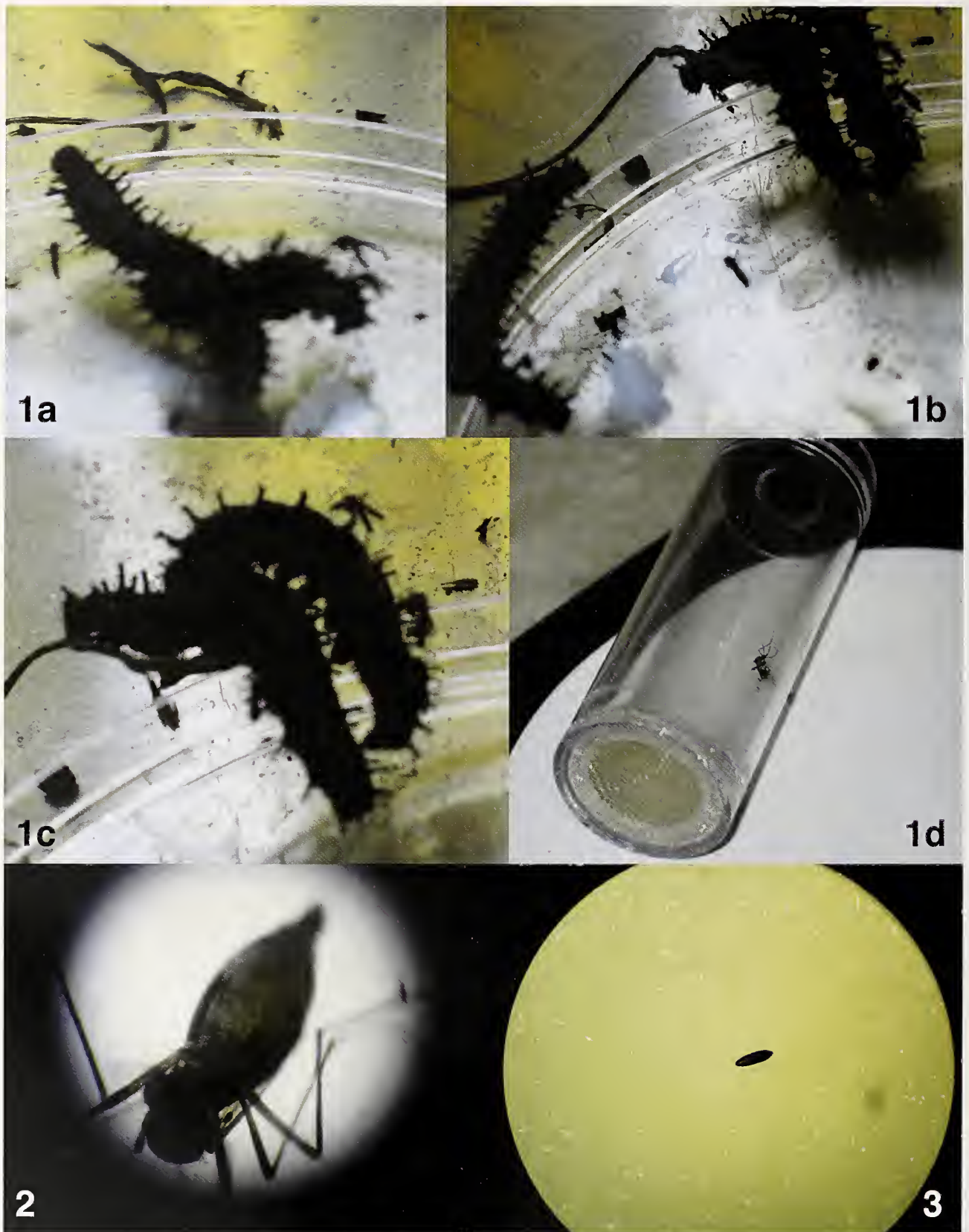
Mosquitoes are thought of as taking blood meals from vertebrate animals exclusively, be they mammals, birds, reptiles, or amphibians. There are even reports of mosquitoes biting fish, although at least one of them is questionable (Sloof & Marks, 1965; Mulhern, 1983). Records of mosquitoes taking meals from invertebrate hosts are far rarer, and Downes (1958) discounted records of mosquitoes feeding on invertebrate hosts. A literature search conducted via Google Scholar and the Armed Forces Pest Management Board Literature Database revealed that, since the publication of Downes' review, only a few authors have published additional accounts of mosquitoes using invertebrate hosts for blood meals (Table 1). According to Harris *et al.* (1969), invertebrate hemolymph meals taken by mosquitoes may have been missed because blood meal analyses generally are done only if the mosquito abdomen is red or dark colored, and then they are only screened against vertebrate antisera. Modern methods of blood meal identification use molecular methods but again, they are screened for vertebrate hosts (e.g. Kent & Norris, 2005). Invertebrate hemolymph generally is clear, yellow, or green; it may be mistaken for a sugar meal and ignored (Rapp, 1947; Harris *et al.*, 1969). Harris & Cooke (1969) stated that the volume of hemolymph taken from caterpillars is about the same as the volume of blood taken from vertebrate hosts; they believed that the quality of the hemolymph meal was inferior to that of the blood meal for purposes of developing eggs.

The Common Buckeye, *Junonia coenia* (Hübner), is found throughout Florida, in much of the southern United States, and into Mexico and the Caribbean (Minno & Emmel, 1993; Daniels, 2003). Three 5th instars (purchased from a butterfly farm) were placed

into a cage containing about 100 female *Aedes aegypti* (L.) mosquitoes reared from larvae collected in the vicinity of Marathon, Florida. The mosquitoes were of varying ages, and had been provided a 10% sucrose solution as adults but never a blood meal. No mosquitoes attempted to feed within 10 minutes. The caterpillars were removed from the cage and "coddled" (*sensu* Harris *et al.*, 1969), i.e., immersed into hot water. This process was repeated three times with the same caterpillars. Every time that the coddled caterpillars were placed into the mosquito cage, numerous female mosquitoes attacked the caterpillars (Fig. 1). Attacks were almost all unsuccessful due to the caterpillars' struggles when the mosquitoes probed them, and the caterpillars' scoli appeared to obstruct attempts to feed. (It should be noted that all previous attempts to feed mosquitoes on caterpillars used larvae that were smooth and without scoli.) At least two female mosquitoes fed on the caterpillars. Both female mosquitoes that took meals from caterpillars subsequently had greenish-yellow liquid in their abdomens. One of the females was captured, photographed, and placed into a rearing chamber along with 10% sucrose and moist filter paper for oviposition (Fig. 2). Three days later the abdomen was no longer engorged. A brown stain was seen on the filter paper, suggesting the meal may have been voided, as *Ae. aegypti* are known to do; females excrete almost 40% of the liquid portion of the blood meal within the first hour after feeding (Gillett, 1956; Beyenbach, 2003). On the fifth day after feeding, the filter paper was removed and examined under a dissecting microscope. Four eggs were found (Fig. 3). The temperature of the laboratory was about 28° C, so finding eggs on the fourth or fifth day was expected (Marchoux *et al.*, 1903). The filter paper was allowed to dry and placed into a plastic bag for two weeks to allow for embryonic development and to maintain humidity. After two weeks, 24.8 ml distilled water was placed into an 8 dram glass vial and 2 mg lactalbumin was added to the water to promote bacterial growth and lower oxygen content. Bacteria serve as a food source for first instar mosquitoes; a low oxygen level in the water is a hatching stimulus for mosquito eggs. Three days were allowed for bacterial growth, after which

Received: 21 July 2014

Accepted: 3 September 2014



Figures 1-3. 1. Mosquitoes feeding on caterpillars (1a, 1b, 1c) and mosquito captured immediately after feeding (1d). 2. Mosquito with hemolymph meal. 3. One of four *Aedes aegypti* eggs laid on filter paper after the mosquito fed on a caterpillar.

Table 1. Literature records of mosquitoes feeding on caterpillars. Key: O = oviposition not reported; N = no viable eggs laid; Y = viable eggs laid.

Lepidopteran	Common Name	Mosquito	Result	Reference
<i>Heliothis subflexa</i> (Guenée)	Subflex Straw Moth	<i>Anopheles stephensi</i> Liston	O	George <i>et al.</i> 2013
<i>Spodoptera littoralis</i> Boisduval	Egyptian Cotton Leafworm	<i>Aedes aegypti</i> (L.)	O	Martel <i>et al.</i> 2011
<i>Hyles euphorbiae</i> (L.)	Spurge Hawk Moth	<i>Aedes aegypti</i> (L.)	Y	Harris <i>et al.</i> 1969
<i>Hyles euphorbiae</i> (L.)	Spurge Hawk Moth	<i>Culex territans</i> Walker	O	Harris <i>et al.</i> 1969
<i>Hyles euphorbiae</i> (L.)	Spurge Hawk Moth	<i>Culex territans</i> Walker	O	Harris & Cooke 1969
<i>Manduca quinquemaculata</i> Haworth	Five-spotted Hawk Moth	<i>Aedes aegypti</i> (L.)	N	Harris <i>et al.</i> 1969
<i>Calophasia lunula</i> (Hufnagel)	Toadflax Brocade Moth	<i>Aedes aegypti</i> (L.)	Y	Harris <i>et al.</i> 1969
<i>Euxoa messoria</i> (Harris)	Darksided Cutworm	<i>Aedes aegypti</i> (L.)	Y	Harris <i>et al.</i> 1969
<i>Danaus plexippus</i> (L.)	Monarch Butterfly	<i>Aedes aegypti</i> (L.)	N	Harris <i>et al.</i> 1969
<i>Galleria mellonella</i> (L.)	Greater Wax Moth	<i>Aedes aegypti</i> (L.)	Y	Harris <i>et al.</i> 1969

the mosquito eggs were placed into the water. Two of the eggs hatched within an hour. Four days later, a third egg had hatched. All three larvae pupated and 2 male and 1 female adult mosquitoes emerged from the pupae.

Harris *et al.* (1969) and Martel *et al.* (2011) both ask a critically important question: does this phenomenon occur in nature? If so, it could have great impacts on lepidopteran biology. Martel *et al.* (2011) reported that in the presence of mosquitoes, larvae of the noctuid moth *S. littoralis* Boisduval had a longer development time, lower pupal weight, spent less time feeding and more time trying to move away from mosquitoes than did caterpillars not exposed to mosquitoes. Time spent fleeing mosquitoes is time spent not feeding, exposure to predators is greater, and there is the risk of not finding a suitable host plant again (Martel *et al.*, 2011). There is also the unanswered question of whether mosquitoes transmit diseases to lepidopteran larvae (Martel *et al.*, 2011). George *et al.* (2013) reported that *Anopheles stephensi* Liston attacks and feeds on living and dead larvae of another species of noctuid moths, viz., *Heliothis subflexa* (Guenée). Interestingly, this mosquito is attracted to caterpillars that are infected with the fungal pathogen *Beauveria bassiana*. Does *An. stephensi* serve as a vector of the pathogen to caterpillars? Or, do caterpillars serve as reservoirs for mosquito pathogens? Martel *et al.* (2011) reported that mosquitoes that fed on infected caterpillars themselves became infected with *B. bassiana*. In another study, all five mosquitoes that were fed on nuclear polyhedrosis-infected *H. euphorbiae* larvae died after feeding (Harris & Cooke, 1969). It may be that caterpillars serve as reservoirs for mosquito pathogens. If so, this could prove to be of importance to agriculture and public health.

Another question that remains is whether the hemolymph meal affects the mosquito that ingests it. Harris *et al.* (1969) raised the question: do toxic compounds sequestered by lepidopteran larvae interfere with mosquito egg development? Harris & Cooke (1969) observed that most mosquitoes that fed on monarch larvae died within three days. This was attributed to sequestration of toxins by the monarch butterfly larvae. Buckeye larvae sequester iridoid glycosides from their host plants (Bowers & Collinge, 1992). When present in the hemolymph, these compounds are repellent to predators (De la Fuente *et al.*, 1995).

Yet another consideration is the value of lepidopteran hemolymph as a food for mosquitoes. Harris & Cooke (1969) considered hemolymph to be an inferior food for mosquitoes, and they reported lower rates of follicular maturation and egg development. In the present instance, only four eggs were laid, whereas the usual number is between 100 and 200 eggs per vertebrate blood meal (Zettel & Kaufman, 2009).

Still one more question remains: why would mosquitoes take a hemolymph meal from caterpillars? Harris & Cooke (1969) thought that invertebrate prey could represent a food source of last resort, when no vertebrate hosts were available and eggs required a protein source for maturation. Martel *et al.* (2011) speculated that this might be one explanation, but also suggested two other possibilities. First, that under poor weather conditions mosquitoes and caterpillars might seek shelter in the same places and thus it would be easier and safer to exploit caterpillars than to leave the shelter to seek a vertebrate host. The other is that this behavior represents an ancestral feeding behavior that mosquitoes have not lost due to a lack of selective pressure against it.

In his study on the effects of mosquito control pesticides on butterfly populations in the Florida Keys, Salvato (2001) reported that, at least in some instances, butterfly numbers were greater in mosquito control-treated areas than in non-treated areas. A variety of larvicides and adulticides is used for mosquito control in Florida (Rey *et al.*, 2012). It is tempting to jump to the conclusions that mosquitoes may negatively impact caterpillars or that mosquito control operations may benefit caterpillars. The intersection of mosquito control and lepidopteran conservation is a fertile ground for investigation and controversy. It is in the hope of stimulating the former instead of the latter that the preceding observations and photographs are offered.

LITERATURE CITED

- BEYENBACH, K.W. 2003. Transport mechanisms of diuresis in Malpighian tubules of insects. *Journal of Experimental Biology* 206: 3845-3856.
- BOWERS, M.D. & S.K. COLLINGE. 1992. Fate of iridoid glycosides in different life stages of the Buckeye, *Junonia coenia* (Lepidoptera, Nymphalidae). *Journal of Chemical Ecology* 18: 817-831.
- DANIELS, J.C. 2003. *Butterflies of Florida*. Field guide. Adventure Publications, Cambridge, MN. 256 pp.
- DE LA FUENTE, M., L. DYER, & M.D. BOWERS. 1995. Catalpol in the hemolymph of Buckeye larvae (*Junonia coenia* Nymphalidae) acts as a deterrent to ants. *Chemoecology* 5/6: 13-18.
- DOWNES, J.A. 1958. The feeding habits of biting flies and their significance in classification. *Annual Review of Entomology* 3: 249-266.
- GEORGE, J., N.E. JENKINS, S. BLANFORD, M.B. THOMAS, & T.C. BAKER. 2013. Malaria mosquitoes attracted by fatal fungus. *PLoS ONE* 8(5): e62632. doi:10.1371/journal.pone.0062632
- GILLET, J.D. 1956. Initiation and promotion of ovarian development in the mosquito *Aedes (Stegomyia) aegypti* (Linnaeus). *Annals of Tropical Medicine and Parasitology* 50: 375-380.
- HARRIS, P. & D. COOKE. 1969. Survival and fecundity of mosquitoes fed on insect haemolymph. *Nature* 222: 1264-1265.
- HARRIS, P., D.F. RIORDAN, & D. COOKE. 1969. Mosquitoes feeding on insect larvae. *Science* 164: 184-185.
- KENT, R.J. & D.E. NORRIS. 2005. Identification of mammalian blood meals in mosquitoes by a multiplexed polymerase chain reaction targeting cytochrome b. *American Journal of Tropical Medicine and Hygiene* 73: 336-342.
- MARCHOUX, E., A. SALIMBENI, & P.L. SIMOND. 1903. La fièvre jaune. Rapports de la Mission Française. *Annales de l'Institut Pasteur* 17: 665-731.
- MARTEL, V., F. SCHLYTER, R. IGNELL, B.S. HANSSON, P. ANDERSON. 2011. Mosquito feeding affects larval behaviour and development in a moth. *PLoS ONE* 6(10): e25658. doi:10.1371/journal.pone.0025658
- MINNO, M.C. & T.C. EMMEL. 1993. *Butterflies of the Florida Keys*. Scientific Publishers. Gainesville, FL. vii + 168 pp.
- MULHERN, T.D. 1983. Do mosquitoes feed on trout? *Mosquito News* 43: 235-236.
- RAPP, J.L.C. 1947. Insect hemolymph: a review. *Journal of the New York Entomological Society* 55: 295-308.
- REY, J.R., W.E. WALTON, R.J. WOLFE, R. CONNELLY, S.M. O'CONNELL, J. BERG, G.E. SAKOLSKY-HOOPES, A.D. LADERMAN. 2012. North American wetlands and mosquito control. *International Journal of Environmental Research and Public Health* 9: 4537-4605.
- SALVATO, M.H. 2001. Influence of mosquito control chemicals on butterflies (Nymphalidae, Lycaenidae, Hesperidae) of the Lower Florida Keys. *Journal of the Lepidopterists' Society* 55: 8-14.
- SLOOF, R. & E.N. MARKS. 1965. Mosquitoes (Culicidae) biting a fish (Periophthalmidae). *Journal of Medical Entomology* 2: 16.
- ZETTEL, C. & P. KAUFMAN. 2009. Yellow fever mosquito: *Aedes aegypti* (Linnaeus) (Insecta: Diptera: Culicidae). University of Florida IFAS Extension Publication EENY 434. 8 pp.

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